MECHANISM OF VITAMIN B₆-ENZYME INHIBITION BY HORMONES

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Recently, many studies on the relations of hormones to vitamins have been made. Especially regarding the relation of vitamin B₆ (B₆) to hormones, it has been observed that the functions of anterior pituitary (1, 2), adrenal glands (3), thyroid glands (4) and sexual glands (5, 6) are depressed in vitamin B₆-deficient rats, whereas the symptoms of B₆ deficiency are aggravated after administration of growth hormone (7) or L-thyroxine (8). The mechanisms of action of these hormones, however, have been insufficiently solved. Harvath (8) had suggested the possibility of Snell's theory (9) of the formation of a Schiff's base between L-thyroxine and pyridoxal-5-phosphate (10) for the development of B₆-deficiency after administering L-thyroxine. It was reported previously by the authors (11) that growth hormone inhibited the tryptophanase of *Escherichia coli communior*. To clarify the mechanism of inhibition by these hormones of tryptophanase which is relatively convenient for treatment and is considered to need a metal and thiol for full activity, the effects of various substances on the inhibition caused by several hormones are investigated.

EXPERIMENTAL

Tryptophanase — *Escherichia coli communior* which had been previously adapted to L-tryptophan was lyophilized, triturated with quartz sand and percolated in physiological sodium chloride solution. The percolate was adjusted to pH 5.0 with 10 M acetic acid and centrifuged off. The precipitate was dissolved in M/15 phosphate buffer (pH 7.8) and dialyzed for 24 hr at 5°. It was used as an enzyme solution which contained 0.4 mg protein per ml.

Chemicals — Pyridoxal-5-phosphate (PAL-P), growth hormone, thyroxine and reduced glutathione (GSH) were the preparations of Nutritional Biochemical Corporation. Parotin (hormone of salivary glands) was the preparation of Teikoku Zoki KK.

Reaction System — It consisted of 0.5 ml of tryptophanase solution, 1.0 ml of M/15 phosphate buffer (pH 7.8), 0.1—2.0 μ g of PAL-P, 0.05—0.6 μ moles of glutathione, 5—50 μ moles of NH₄Cl and 0.1—2.0 mg of each hormones in the

total volume of 2.0 ml.

Determination of Enzyme Activity—The above solution was preincubated at 37° for 10 min. After adding 0.4 ml of *M*/160 tryptophan solution, the mixture was incubated further for 10 min. After deproteinization by 100 g per 100 ml of trichloroacetic acid, the indole produced was colorimetrically determined according to Goto (12) and the pyruvic acid produced was also colorimetrically determined by the method of Friedman and Haugen (13).

RESULTS

TABLE I
Inhibition of Tryptophanase by Various Hormones
Reaction system, see the text.

Hormone	Dose	Indole formed	Pyruvic acid formed
	mg	μg	μg
Parotin	0	23.0	14.5
	0.1	22.4	14.3
	0.3	14.6	9.8
	0.5	10.0	6.2
	0.8	3.0	1.9
	1.0	0	0
Growth hormone	0	23.0	14.5
	0.2	21.3	11.5
	0.5	13.0	8.3
	1.0	2.7	1.7
	1.5	0	0
Thyroxine	0	23.0	14.5
	0.3	19.5	13.2
	0.5	13.2	8.3
	1.0	4.0	2.8
	2.0	0	0

FIG. 1 Restoration by Pyridoxal Phosphate of Tryptophanase Inhibition

As presented in Table I, appreciable parallelism was found between indole and pyruvic acid when parotin, growth hormone and thyroxine were used as test hormones, and the inhibition of tryptophanase was dependent in each case on the concentration of these hormones. The enzyme was completely inhibited by 1.0 mg of parotin, 1.5 mg of growth hormone or 2.0 mg of thyroxine in this condition but it was not inhibited by adrenocorticotrophic hormone (ACTH), hydrocortisone and insulin. Further, restoration of the inhibition by PAL-

P, a coenzyme of tryptophanase, was observed. As shown in Table II and Fig. 1, the inhibitions by growth hormone and parotin were not recovered by the addition of PAL-P, the percentage inhibition being invariable. On

TABLE II

Effect of Pyridoxal Phosphate on Tryptophanase Inhibition by Hormones

Reaction system: The same as in Table I except those given in this Table. Parenthesized figures indicate percentage inhibition. Also the same in the following Tables.

Reaction mixture			Indole produced (μg)			
PAL-P	GSH	NH ₄ Cl	No inhibitor	Parotin (0.5 mg)	Growth hormone (0.5 mg)	Thyroxine (0.5 mg)
μg	μmole	μmoles				
0.1	0.1	10	4.2	1.8 (57.1)	1.9 (44.8)	1.2 (71.5)
0.3	0.1	10	9.7	4.6 (52.3)	5.5 (46.7)	4.0 (58.6)
1.0	0.1	10	23.0	10.0 (56.5)	13.0 (43.5)	13.2 (42.6)
5.0	0.1	10	72.8	36.4 (53.6)	38.5 (47.0)	58.4 (19.7)
20.0	0.1	1	71.9	33.1 (54.0)	40.0 (43.8)	60.7 (7.2)

the contrary, in thyroxine, the effect of PAL-P on the restoration of inhibition was very distinct.

FIG. 2 Restoration by Ammonium Ion of Tryptophanase Inhibition

FIG. 3 Restoration by Glutathione of Tryptophanase Inhibition

The data showing the effect of ammonium ion are presented in Table III and Fig. 2. The inhibitions by growth hormone and parotin were reversed by addition of ammonium ion, whereas the percentage inhibition by thyroxine remained practically invariable. On the other hand, glutathione had greater influence on the activity of the enzyme (Table IV and Fig. 3) than ammonium ion. The inhibitions by growth hormone and parotin were restored completely by 0.3–0.6 μmoles of glutathione, but the inhibition by thyroxine remained unchanged.

TABLE III
Effect of Ammonium Ion on Tryptophanase Inhibition by Hormones

Reaction mixture			Indole produced (μg)			
PAL-P	GSH	NH ₄ Cl	No in- hibitor	Parotine (0.5 mg)	Growth hormone (0.5 mg)	Thyroxine (0.5 mg)
μg	μmole	μmoles				
1	0.1	5	12.7	2.4 (81.1)	5.2 (62.3)	7.2 (43.4)
1	0.1	10	23.0	10 (56.5)	13.0 (43.5)	13.2 (42.6)
1	0.1	20	43.0	30.7 (29.2)	31.1 (27.5)	26.1 (39.0)
1	0.1	50	67.2	54.0 (19.5)	57.0 (14.8)	36.9 (45.0)

TABLE IV
Effect of Reduced Glutathione on Tryptophanase Inhibition by Hormones

Reaction mixture			Indole produced (μg)			
PAL-P	GSH	NH ₄ Cl	No in- hibitor	Parotine (0.5 mg)	Growth hormone (0.5 mg)	Thyroxine (0.5 mg)
μg	μmole	μmoles				
1	0.05	10	11.8	2.8 (76.3)	5.0 (57.6)	6.5 (44.9)
1	0.1	10	23.0	10.0 (56.5)	13.0 (43.5)	13.2 (42.5)
1	0.3	10	49.2	38.5 (21.6)	49.2 (0)	31.5 (35.9)
1	0.6	10	72.3	72.3 (0)	72.3 (0)	42.0 (41.9)

DISCUSSION

In the cases of growth hormone and parotin, PAL-P concentration has no effect on the inhibition by these hormones. However, this inhibition was restored by the addition of glutathione (more than 0.3 μmoles) completely, and also by a large quantity of ammonium ion (more than 50 μmoles) as well. This suggests that the mechanism of inhibition does not consist in the combination or competition of the hormones with the coenzyme, but that the hormones act on the apoenzyme, especially they inactivate the thiol group, which is assumed to be the active center of the apoenzyme. Thus, a small quantity of glutathione can protect the group completely. Ammonium ion, on the other hand, is surmised to participate in electronic transposition.

The inhibition of thyroxine, on the contrary, is not affected by reduced glutathione or ammonium ion, but is restored by a high concentration of PAL-P. This fact suggests that the inhibition by thyroxine consists in the mechanism, in which the hormone either competes with the coenzyme for the apoenzyme or combines with the coenzyme, forming a Schiff's base.

Recently, a purified amino acid decarboxylase from rat liver has been shown to need PAL-P for the activity together with a thiol group and metal ion. Assuming that the above finding is also applicable to the B₆-enzymes of mammals, B₆-enzymes may possibly be controlled either through phosphate group or through sulfhydryl group and metal ion, thus participating in the metabolism of amino acids and proteins.

SUMMARY

The mechanism of inhibition of *Escherichia coli communior* tryptophanase by thyroxine, parotin and growth hormone was considered in relation to apoenzyme, metal ion and sulfhydryl group. Each hormone was found to show its own different attitude of inhibition.

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